Effects of soil fungi, disturbance and propagule pressure on exotic plant recruitment and establishment at home and abroad

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Summary

1. Biogeographic experiments that test how multiple interacting factors influence exotic plant abundance in their home and recipient communities are remarkably rare. We examined the effects of soil fungi, disturbance and propagule pressure on seed germination, seedling recruitment and adult plant establishment of the invasive *Centaurea stoebe* in its native European and non-native North American ranges.

2. *Centaurea stoebe* can establish virtual monocultures in parts of its non-native range, but occurs at far lower abundances where it is native. We conducted parallel experiments at four European and four Montana (USA) grassland sites with all factorial combinations of ± suppression of soil fungi, ±disturbance and low versus high knapweed propagule pressure [100 or 300 knapweed seeds per 0.3 m × 0.3 m plot (1000 or 3000 per m^2)]. We also measured germination in buried bags containing locally collected knapweed seeds that were either treated or not with fungicide.

3. Disturbance and propagule pressure increased knapweed recruitment and establishment, but did so similarly in both ranges. Treating plots with fungicides had no effect on recruitment or establishment in either range. However, we found: (i) greater seedling recruitment and plant establishment in undisturbed plots in Montana compared to undisturbed plots in Europe and (ii) substantially greater germination of seeds in bags buried in Montana compared to Europe. Also, across all treatments, total plant establishment was greater in Montana than in Europe.

4. *Synthesis.* Our results highlight the importance of simultaneously examining processes that could influence invasion in both ranges. They indicate that under 'background' undisturbed conditions, knapweed recruits and establishes at greater abundance in Montana than in Europe. However, our results do not support the importance of soil fungi or local disturbances as mechanisms for knapweed's differential success in North America versus Europe.

Key-words: biogeography, *Centaurea stoebe*, disturbance, exotic invasion, germination, invasion ecology, propagule pressure, soil fungal pathogens

Introduction

Biological invasions remain an ecological enigma. On one hand, most species establishing in new regions only colonize at low densities (Williamson 1996). These species increase local diversity but owing to their low abundance they otherwise play a minor role in recipient communities (Ortega & Pearson 2005). On the other hand, some non-native species attain astoundingly high abundances where they are introduced, and their abundance can lead to profound impacts on recipient communities and ecosystems (Liao *et al.* 2008; Vilà *et al.* 2011). The factors that enable these strong invaders to become so successful, despite often being fairly inconspicuous where they are native, remain elusive (Shea & Chesson 2002).

Many hypotheses have been advanced for exotic success (Elton 1958; Callaway & Aschehoug 2000; Mack *et al.* 2000; Müller-Schärer, Schaffner & Steinger 2004; Hierro, Maron &

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Callaway 2005; Kulmatiski *et al.* 2008). Most of these hypotheses share a fundamental attribute: they posit that some biological processes act in a fundamentally different way in a species' native range than in its introduced range. For example, the 'enemy escape' hypothesis poses that specialist pathogens or herbivores strongly suppress exotic plant population growth or abundance at home. In non-native communities, the relaxation of this limiting factor is thought to drive positive population growth or greatly enhanced abundance compared to the native range (Elton 1958; Maron & Vilà 2001).

Our thinking about the factors responsible for the differential success of invasives in their native versus non-native ranges derives in part from our knowledge of how particular processes influence plant abundance in general (e.g. Gurevitch *et al.* 2011). For example, much work has demonstrated that removal of competitors by disturbance, increasing propagule pressure or soil pathogen suppression can have a positive effect on the recruitment and/or establishment of native plants (Bell, Freckleton & Lewis 2006; MacDonald & Kotanen 2010) as well as exotics (Hobbs 1989, 1991; Hobbs & Huenneke 1992; D'Antonio, Dudley & Mack 1999; Mack *et al.* 2000; Parker 2001; Kellogg & Bridgham 2004; Paiaro, Mangeaud & Pucheta 2007; Britton-Simmons & Abbott 2008; Simberloff 2009). Yet, for these factors to explain biogeographic patterns of invasiveness, disturbance or a given level of propagule pressure must have a substantially greater influence on a plant's population growth or abundance within recipient communities compared to its native community (Hierro, Maron & Callaway 2005). Unfortunately, such comparative information is rare. We know of only two field studies that have examined the impacts of propagule pressure, disturbance and other factors on the recruitment of plants in their native and introduced range (Hierro *et al.* 2006; Williams, Auge & Maron 2010). Similarly, while we know that specialist enemies including some soil pathogens can negatively influence seedling survival and plant performance (van der Putten, van Dijk & Peters 1993; Bever 1994; Packer & Clay 2000; Klironomos 2002; Reinhart *et al.* 2005; Bell, Freckleton & Lewis 2006; van der Heijden, Bardgett & van Straalen 2008; Kulmatiski *et al.* 2008; Mordecai 2011), we lack comparisons of the relative impact of plant–pathogen interactions for a given species in both its home and recipient communities (but see Reinhart *et al.* 2003; Callaway *et al.* 2004a, 2011a).

The effects of multiple interacting factors on the distribution and abundance of species are increasingly recognized in ecology, but how variation in synergistically interacting mechanisms might favour dramatic variation in the abundance of the same species in native and non-native ranges is generally unknown (van Kleunen *et al.* 2010). Our goal here is to contrast the impacts of multiple, potentially interacting factors in both ranges that have been shown in isolation to inhibit native performance (soil pathogens) or enhance exotic performance (disturbance, propagule pressure). Our intention was to determine whether a strong biogeographic difference in one or more of these factors, or interactions among these factors, might explain invasion success. As well, we were interested in whether these factors might interact in different ways in home versus recipient communities to differentially influence plant abundance. There are only a handful of field studies that have employed parallel experiments within native and introduced populations to test whether natural enemies or disturbance might differentially influence the success of an exotic in its home and recipient community (Reinhart *et al.* 2003; DeWalt, Denslow & Ickes 2004; Hierro *et al.* 2006; Williams, Auge & Maron 2010; Callaway *et al.* 2011a). For example, DeWalt, Denslow & Ickes (2004) established one of the first biogeographic experiments that involved comparing herbivore and pathogen impacts on plant fitness in both the native and introduced range. They found that herbivores and pathogens together greatly reduced the survival of the neotropical shrub, *Clidemia hirta*, in understorey habitats in its native range in Costa Rica, but not in its introduced range in Hawaii. Hierro *et al.* (2006) found that the invasive plant, *Centaurea solstitialis*, responded more positively to disturbance in Argentina and California (where it has been introduced) compared to Turkey (where it is native). Since they also found that Eurasian soil microbes suppressed the growth of *C. solstitialis* more than Californian and Argentinean soil biota did, they suggested that escape from pathogens might explain the biogeographic difference in how *C. solstitialis* responded to disturbance. Finally, by combining manipulative experiments with detailed demography and demographic modelling, Williams, Auge & Maron (2010) found that small-scale disturbances had a greater impact on *Cynoglossum officinale* population growth in the introduced range in Montana compared to the native range in Europe.

We conducted identical experiments in Europe and North America, asking how soil fungi, disturbance and propagule pressure individually and interactively influenced seedling recruitment and adult plant establishment of spotted knapweed (hereafter knapweed), *Centaurea stoebe*. We also examined biogeographical differences in *C. stoebe* germination, and impacts of soil fungi on seed survival. In the intermountain west of North America, knapweed is a potent invader in grassland and understorey habitats, where it can grow at extremely high abundance and form virtual monocultures (Jacobs, Sheley & Cater 2000; Ridenour & Callaway 2001; Ortega & Pearson 2011). Knapweed was likely introduced into North America multiple times (Marrs, Sforza & Huffbauer 2008), and it appears to be an especially good competitor against many of the native perennial plants that inhabit these grasslands (Maron & Marler 2008a; Callaway *et al.* 2011b). At high abundance knapweed significantly alters the composition of native communities and their productivity (Maron & Marler 2008b). In contrast, in its native grassland habitats in Europe and Eurasia, knapweed appears to occur at much lower densities (i.e. it seldom grows in large monocultures as it does in some portions of the west) and its competitive impacts are reduced compared to areas in North America (Callaway *et al.* 2011b).

Materials and methods

Our experiments took place across 8–10 grassland sites (depending on experiment; Table 1), 4–5 in Europe where knapweed is native

Table 1. Name of experimental sites, State or Country where sites are located, site latitude and longitude, year in which experiments were initiated, experiments initiated at each site (SA = seed addition, BB = buried seed bags, FA = soil sampled for fungicide assay) and elevation of each site

Site	State/Country	Lat/Long	Year	Experiment	Elevation (m)
Ninemile	Montana	46°56′15.1″ N 113° 27′24.0″ W	2007	SA, BB, FA	1126
Davis	Montana	46°43′52.7″ N 114° 02′ 53.9″ W	2007	SA, BB, FA	993
Grant	Montana	46° 56′07.7″ N 114° 01′06.6″ W	2008	SA, BB, FA	1106
Elk Park	Montana	47° 09′52.7″ N 114° 07′39.66″ W	2007	BB, FA	1280
Perma	Montana	47° 22′04.8″ N 114° 33′34.5″ W	2009	SA, FA	796
Gont	Switzerland	47°16′56.74″ N 8°08′47.45″ E	2007	SA, BB, FA	519
Biere	Switzerland	46°31′29.19″ N 6°19′41.84″ E	2007	BB, FA	697
Perieni	Romania	46° 16′ 33.8″ N 27° 37′ 24.1″ E	2007	SA, BB, FA	164
David's	Romania	47° 11′ 47.2″ N 27° 28′ 03.5″ E	2007	SA, BB, FA	110
Mariagyud	Hungary	45° 52′ 47.4″ N 18° 14′ 50.8″ E	2008	SA, BB, FA	157

and 4–5 in Montana, USA where knapweed is an exotic invader. Knapweed can grow at high abundance over large areas in western Montana, but we chose locations where it occurred at moderate-to-low densities and where the background plant community was composed mostly of native plants. We avoided sites that either currently supported dense knapweed stands or had a legacy of high knapweed occupancy, since these sites often have a copious knapweed seed bank, a severely suppressed native community, and potentially altered soil biota (*sensu* Kulmatiski, Beard & Stark 2006), factors that could confound direct comparisons of knapweed's performance in North America versus Europe. In Europe, we worked at sites where knapweed is known to be a tetraploid, the same ploidy level as are plants that occur in Montana (Treier *et al.* 2009; Mráz *et al.* 2011).

At each grassland site, we haphazardly established and permanently marked 96 0.3 × 0.3 m plots and randomly assigned one treatment from a fully factorial combination of ± disturbance, 0, 100 or 300 seed addition and ± soil fungicide treatment to each plot (each treatment combination was replicated eight times except in Hungary where there were six replicates per treatment). We chose seed densities to span a range that might represent 'low' or 'high' propagule pressure and are within the range of seed densities that can be produced around large adult plants (Watson & Renney 1974). Plots were spaced at least 1 m apart. For plots assigned to receive '+disturbance', we used a hoe to remove all above-ground vegetation, disturbing soil to a depth of 15 cm, breaking up large chunks of soil and mixing vegetation and litter with the soil to attain a uniform flat surface. This effectively removed competitors and simulated small-scale disturbances that might occur naturally. After the first year of seedling recruitment, we weeded all disturbed plots for the duration of the experiment to keep them free of vegetation other than knapweed. Our goals in doing this were twofold: first, we wanted to maintain a relatively competition-free environment across years, so that any seedlings that germinated after the initial year of seed addition would experience a low competition environment similar to that experienced by seedlings that germinated in year 1. We could not entirely mimic the initial effects of disturbance after year 1 since this would entail turning over the soil and killing existing plants. Second, by keeping disturbed plots free of vegetation, we could determine how interspecific competition influenced the survival of knapweed plants that established in our plots. We defined establishment as survival beyond the first summer. We weeded disturbed plots early in spring when young plants could be easily removed without harming young knapweed seedlings. We pulled plants out by the roots, but trimmed more substantially rooted plants to ground-level with clippers.

Seed addition plots received either 100 or 300 knapweed seeds that were collected locally at each site. Seeds were added to plots in September of the year in which the experiment was initiated at each site (Table 1). We also established plots to which we added no seeds, so we could assess the extent to which knapweed recruitment out of an existing seed bank influenced recruitment in plots to which we added seeds. Seeds added to fungicide-treated plots were first treated with the fungicide Maxim XL to suppress fungal pathogens that might attack seeds before germination. Maxim is a combination of fludioxonil and mefenoxam, which controls soil-borne and seed-borne diseases such as *Pythium* and *Phytophthora*. We mixed a slurry of 5.32 mL of Maxim XL and 118 mL of water. With a pipette, we added 0.1 mL to containers containing 100 seeds and 0.2 mL to containers containing 300 seeds. We shook each container to coat all seeds, and then left the containers open for several hours to allow seeds to dry.

Plots receiving the '+fungicide' treatment received a soil drench consisting of a mix of two fungicides, Thiophanate methyl 500SC (United Phosphorus Inc., King of Prussia, PA, USA) and Ridomil Gold EC (Syngenta Corporation, Wilmington, DE, USA). Thiophanate methyl 500SC is a broad-spectrum fungicide that suppresses pathogenic fungi in the genus *Fusarium*. Several bacteria degrade this fungicide relatively rapidly in soil (Cycón, Wójcik & Piotrowska-Seget 2011). It contains no phosphorus and four atoms of nitrogen (chemical composition: $C_{12}H_{14}N_4O_4S_2$). Ridomil Gold is also a systemic fungicide (active ingredient is mefenoxam, a synthetic isomer of metalaxyl) that controls diseases caused by Oomycete fungi, particularly Pytheaceous fungi. It also contains no phosphorus and only has one atom of nitrogen (chemical composition: $C_{15}H_{21}NO_4$). Ridomil Gold has been successfully used in other ecological experiments (Bell, Freckleton & Lewis 2006). Mefonoxam has low toxicity to organisms other than fungi and does not have strong inhibitory effects on arbuscular-mycorrhizal fungi (AMF) in agricultural systems (Afek, Menge & Johnson 1990; Seymour, Thompson & Fiske 1994). However, we cannot be certain that these fungicides did not suppress non-pathogenic as well as pathogenic fungi. For example, the reduction in adult plants that established in fungicide versus control plots in Europe (although not in the U.S.; see Results) is contrary to what we would expect if the sole result of fungicide application was to reduce knapweed pathogens. Ridomil Gold plus (a formulation we did not use that contains copper oxide 60%) has been found to influence soil acid and alkaline phosphatase activity and increase soil N and organic P mineralization (Demanau *et al.* 2004). Using a backpack sprayer, we applied a 0.11 L water-fungicide soil drench (1 g Cleary's 3336/L H_2O and 0.117 mL Ridomil Gold EC/L H_2O) to each '+fungicide'

plot in late April/early May and again in late June/early July each year. Immediately after spraying the fungicide mixture, we sprayed each plot with 0.33 L of water to incorporate fungicides into the soil. Control plots received 0.44 L of water, also applied to the soil surface with a pressurized backpack sprayer.

To determine the efficacy of our fungicide treatment for reducing fungal pathogens and to examine non-target effects, we established 16 additional plots (in addition to the 96 plots mentioned above; plot size = 0.75 × 0.75 m) adjacent to our seed addition plots at all sites (Table 1). At each site, half of these plots were treated with fungicide and half were watered, in an identical manner as the experimental seed addition plots. In September 2009, we took at least three core samples from the top 15 cm of soil from each of the ± fungicide plots. We pooled soil collected from each plot and then subsampled from this pooled sample for measures of fungal biomass, bacterial biomass and mycorrhizal infectivity. In previous work where this mix of soil fungicides was applied at the same concentrations as above twice yearly to plots for several years, we found no effects of fungicide on non-fungal microbial biomass or plant available nitrogen (Maron *et al.* 2011; J. L Maron and C. Cleveland, unpublished data). We assessed fungal and bacterial biomass with a differential fluorescent staining technique using europium (III) thenoyltrifluoroacetonate and fluorescent brightener (Morris *et al.* 1997; Klironomos, Rillig & Allen 1999). Active fungal hyphae and bacterial cells were viewed under a compound microscope, and images were captured using NIS-Elements 1.0 image analysis software (Nikon Inc., Melville, NY, USA) where the number of bacterial cells and hyphal length were converted to biomass (Morris *et al.* 1997). Mycorrhizal infectivity is a measure of the number of infective mycorrhizal fungal propagules in the soil. The assay uses a standard and highly colonized mycorrhizal host – *Sorghum bicolor* L. – grown as a seedling for 10 days in the presence of a soil sample, and then assesses the number of mycorrhizal infection units that develop in the roots (Klironomos 1995). At harvest, roots were washed free of soil, weighed, cleared and stained with Chlorazol Black E (Brundrett, Piché & Peterson 1984). Infection units per sample were calculated as the percentage of the root system colonized by arbuscular mycorrhizal fungi following 150 random observations at 250× magnification along each root system. To determine whether fungicide application reduced the frequency of pathogenic interactions on these same *Sorghum* roots, we also scored the number of necrotic lesions along 150 random observations at 100× magnification along each root system.

Since knapweed seeds germinate in fall and spring, we censused plots in April/May and September/October. In spring, plots were censused approximately 1–2 weeks after fungicide application. At each census, we counted the total number of newly germinated seedlings (i.e. plants with at least two true leaves that had emerged since the last census) and marked each individual with a coloured plastic toothpick. We measured cumulative seedling 'recruitment' as the total of these counts through time; whereas 'germination' was measured in the mesh bags as described below. After initial recruitment, we monitored seedling survival from individual cohorts across each subsequent season. The number of adult plants was defined as those that were more than 1-year old at the end of the experiment. When plants bolted for the first time (2 years after germination at most sites), in July/August, we measured the height, number of flowers, buds and flowering stalks on each flowering plant.

To examine how fungal soil pathogens influenced the fate of seeds in the soil, we created 7.5 × 7.5 cm bags (mesh size = 56.25 mm^2) made of polyester fabric. Each bag contained 100 knapweed seeds that were collected from local knapweed populations at each site. Before enclosing seeds in mesh bags, we either treated seeds with a fungicidal powder (fungicide = Maxim XL) or left seeds untreated. At each location shown in Table 1, we buried 32 seed bags, 16 with fungicide-treated seed and 16 control bags. Bags were buried 5 cm deep in October 2007 or 2008 (depending on site; Table 1). Sixteen of these buried bags (8 containing seeds that had received fungicide application, 8 with control seeds) were excavated at each site in 2008 and 2009. Upon excavation, seeds were removed from each bag and individually inspected under a dissecting microscope. Seeds were scored as 'good' if they had completely intact endosperm and were hard on all sides. Seeds were scored as 'germinated' if the seed was split open along its suture and either had a radical growing out of the seed or had other evidence for germination.

ANALYSES

We used four-way ANOVA within the PROC GLIMIX module in SAS (version 9.2, Cary, NC, USA) to test how continent (Europe versus North America), propagule pressure (100 vs. 300 seeds per plot), fungicide and disturbance individually and interactively influenced: (i) cumulative seedling recruitment per plot (i.e. the total number of seedlings that recruited regardless of their ultimate fate) across all years of the experiment, (ii) the total number of adult plants that established in plots at the end of the experiment [adult plant(s) are defined as those > 1-year old and include both flowering and non-flowering individuals] and (iii) the total number of flowering plants/plot at the end of the experiment. Due to skew in our data, we used an underlying negative binomial distribution applying the log link function in GLIMIX. Site within continent was treated as a random factor. Since seedling recruitment into plots to which we added no seeds was minimal (mean seedlings recruited into disturbed plots = 0.88 and 1.4 seedlings in Europe and Montana, respectively, mean seedlings recruited into undisturbed plots = 0.08 and 0.48 seedlings, respectively), we did not include the zero seeds added plots in the analysis. In analyses of total plants established per plot and number of flowering plants per plot, we did not use data from the Davis site (Table 1). The Davis site was excluded from these analyses because between the second and third year of the experiments, virtually all plants in all of our plots died rapidly for reasons we could not determine. Nothing like this occurred at any other site.

To explore how fungicide application influenced soil fungal biomass, bacterial biomass, AMF infectivity and pathogenic root lesions on *Sorghum* roots, we used the PROC MIXED module in SAS, with continent (Europe versus North America) and fungicide (and fungicide × continent and fungicide × year interactions) as fixed factors and site within continent as a random factor. Because AMF infectivity and pathogenic root lesion data were skewed, we used an underlying negative binomial distribution using the log link function in GLIMIX. We used a two-way ANOVA within the PROC MIXED module in SAS to explore how continent (Europe versus North America) and fungicide individually and interactively influenced the number of seeds that germinated within excavated buried bags. Site within continent was treated as a random factor. We present least square means ± 1 SE from mixed models throughout.

Results

EFFECTS OF SOIL FUNGICIDE APPLICATION

Treating plots with the fungicide soil drench suppressed fungal biomass (mean fungicide = 1.88, mean control = 3.12; $F_{1,8} = 73.15$, $P < 0.0001$) and reduced pathogenic lesions on

Sorghum roots (mean fungicide 7.34, mean control 14.04; $F_{1,8} = 39.20$, $P = 0.0003$). However, fungicide application had no side effects on bacterial biomass (mean fungicide = 0.085, mean control = 0.085; $F_{1,8} = 0.83$, $P = 0.39$) or AMF infectivity (mean fungicide = 1.79, mean control = 1.71; $F_{1,8} = 0.48$, $P = 0.44$). For all variables measured, there was no significant difference between continents or a significant fungicide × continent interaction ($P > 0.05$).

Despite significantly reducing fungal biomass and root lesions on *Sorghum* test plants, fungicide application had no significant main effects on cumulative knapweed seedling recruitment, establishment of adult knapweed plants or the number of flowering plants either in Europe or Montana (Table 2). However, we did find a significant fungicide × continent interaction on adult knapweed establishment (Table 2). The mean number of adult plants in plots treated with fungicide versus control plots in Europe was 0.69 and 1.23, respectively. In contrast, the mean number of adult plants in plots treated with fungicide versus control plots in Montana was 3.6 and 3.0, respectively.

We found no significant effect of fungicide application on either seed germination within buried bags excavated after one (fungicide main effect: $F_{1,7.1} = 3.66$, $P = 0.10$; continent × fungicide interaction: $F_{1,7.1} = 0.13$, $P = 0.73$) or 2 years (fungicide main effect: $F_{1,7.1} = 3.31$, $P = 0.11$; continent × fungicide interaction: $F_{1,7.1} = 0.87$, $P = 0.38$) or on the number of good ungerminated seeds remaining in each buried bag after burial for one (fungicide main effect: $F_{1,7.2} = 2.24$, $P = 0.18$; continent × fungicide interaction: $F_{1,7.2} = 1.62$, $P = 0.24$) or 2 years (fungicide main effect: $F_{1,7.1} = 2.05$, $P = 0.19$; continent × fungicide interaction; $F_{1,7.2} = 1.04$, $P = 0.34$). We did, however, find that there was significantly higher germination of seeds in buried bags excavated after both 1 year ($F_{1,7} = 15.6$, $P < 0.006$) and 2 years ($F_{1,7} = 12.9$, $P < 0.009$) in North America compared to Europe (Fig. 1).

EFFECTS OF DISTURBANCE AND PROPAGULE PRESSURE

Across continent, fungicide and disturbance, the cumulative number of seedlings recruited into plots was not significantly different between the native and introduced range (Table 2). Increasing seed input from 100 to 300 seeds and disturbing plots significantly enhanced knapweed recruitment across continents (Fig. 2 and Table 2). The mean cumulative number of seedlings that recruited into plots with 100 seeds added was 7.05 recruits (7% of seeds added) compared to 15.9 recruits in the plot that received 300 seeds (5.3% of seeds added). Across continent, fungicide and seed addition treatments, the mean cumulative seedling recruitment into undisturbed plots was 7.5 (7.5% of seeds added) compared to 14.8 (4.9% of seeds added) recruits in disturbed plots. Most notably, the effects of disturbance varied significantly between continents (significant continent × disturbance interaction, Table 2). More specifically, a *post hoc* contrast revealed that cumulative seedling recruitment was significantly greater into undisturbed plots in North America compared to undisturbed plots in Europe (Fig. 2a; $F_{1,18} = 38.5$, $P < 0.001$).

Across continent, fungicide, and seed addition treatments, knapweed abundance (i.e. the number of established adult plants) and the number of flowering plants in each plot at the end of the experiment were two to three times higher in Montana than in Europe (mean abundance Montana = 3.3 ± 0.26, Europe = 0.92 ± 0.26; number flowering plants Montana = 1.05 ± 0.17, Europe = 0.41 ± SE 0.09). As well, knapweed abundance and the number of flowering plants per plot were positively affected by both disturbance and propagule pressure (Table 2). The mean number of plants in undisturbed plots at the end of the experiment was 0.74 (mean flowering = 0.22 ± SE 0.05) compared to 4.1 (mean flowering = 1.95 ± SE 0.24) in disturbed plots. The mean number of adult plants at the end of the experiment in plots

Table 2. Results from a four-way anova testing effects of continent (native range versus introduced range), fungicide application, disturbance and propagule pressure (100 vs. 300 seeds added to each plot) on cumulative seedling recruitment per plot, the number of established plants in each plot at the end of the experiment and the number of flowering plants per plot at the end of the study

	Cumulative recruitment	Adult plants	Flowering plants
Continent	$F_{1,42} = 2.36$, $P = 0.13$	$F_{1,35} = 9.57$, $P =$ **0.004**	$F_{1,35} = 11.31$, $P <$ **0.002**
Disturbance	$F_{1,42} = 26.19$, $P <$ **0.001**	$F_{1,35} = 97.57$, $P =$ **0.001**	$F_{1,35} = 87.77$, $P <$ **0.001**
Fungicide	$F_{1,42} = 0.13$, $P = 0.72$	$F_{1,35} = 1.38$, $P = 0.25$	$F_{1,35} = 0.07$, $P = 0.79$
Propagule pressure	$F_{1,42} = 38.01$, $P <$ **0.001**	$F_{1,35} = 26.85$, $P <$ **0.001**	$F_{1,35} = 10.65$, $P <$ **0.003**
Continent × Disturbance	$F_{1,42} = 7.30$, $P <$ **0.010**	$F_{1,35} = 22.43$, $P <$ **0.001**	$F_{1,35} = 18.86$, $P <$ **0.001**
Continent × Fungicide	$F_{1,42} = 0.06$, $P = 0.81$	$F_{1,35} = 4.92$, $P <$ **0.04**	$F_{1,35} = 0.28$, $P = 0.60$
Continent × Propagule pressure	$F_{1,42} = 0.24$, $P = 0.63$	$F_{1,35} = 6.61$, $P <$ **0.02**	$F_{1,35} = 3.44$, $P = 0.072$
Disturbance × Fungicide	$F_{1,42} = 0.31$, $P = 0.58$	$F_{1,35} = 0.13$, $P = 0.72$	$F_{1,35} = 0.19$, $P = 0.66$
Disturbance × Propagule pressure	$F_{1,42} = 3.66$, $P = 0.062$	$F_{1,35} = 0.01$, $P = 0.97$	$F_{1,35} = 0.09$, $P = 0.77$
Fungicide × Propagule pressure	$F_{1,42} = 0.57$, $P = 0.45$	$F_{1,35} = 0.31$, $P = 0.58$	$F_{1,35} = 0.04$, $P = 0.83$
Continent × Disturbance × Fungicide	$F_{1,42} = 0.48$, $P = 0.49$	$F_{1,35} = 1.89$, $P = 0.18$	$F_{1,35} = 0.78$, $P = 0.38$
Continent × Disturbance × Propagule pressure	$F_{1,42} = 1.24$, $P = 0.27$	$F_{1,35} = 4.78$, $P <$ **0.04**	$F_{1,35} = 7.38$, $P <$ **0.011**
Continent × Fungicide × Propagule pressure	$F_{1,42} = 0.01$, $P = 0.94$	$F_{1,35} = 0.48$, $P = 0.49$	$F_{1,35} = 0.05$, $P = 0.82$
Fungicide × Disturbance × Propagule pressure	$F_{1,42} = 0.14$, $P = 0.71$	$F_{1,35} = 0.04$, $P = 0.85$	$F_{1,35} = 0.09$, $P = 0.76$
Continent × Dist. × Fungicide × Propagule pressure	$F_{1,42} = 0.52$, $P = 0.47$	$F_{1,35} = 0.59$, $P = 0.45$	$F_{1,35} = 0.04$, $P = 0.85$

Bolded *P*-values are those where $P < 0.05$.

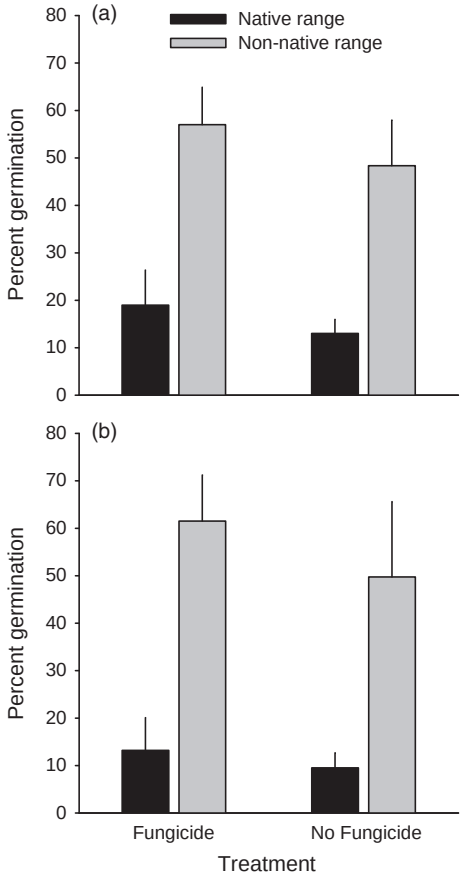

Fig. 1. Effects of continent of burial and fungicide application on mean (+SEM) percentage of knapweed seeds that germinated in buried bags after (a) 1 year and (b) 2 years of burial.

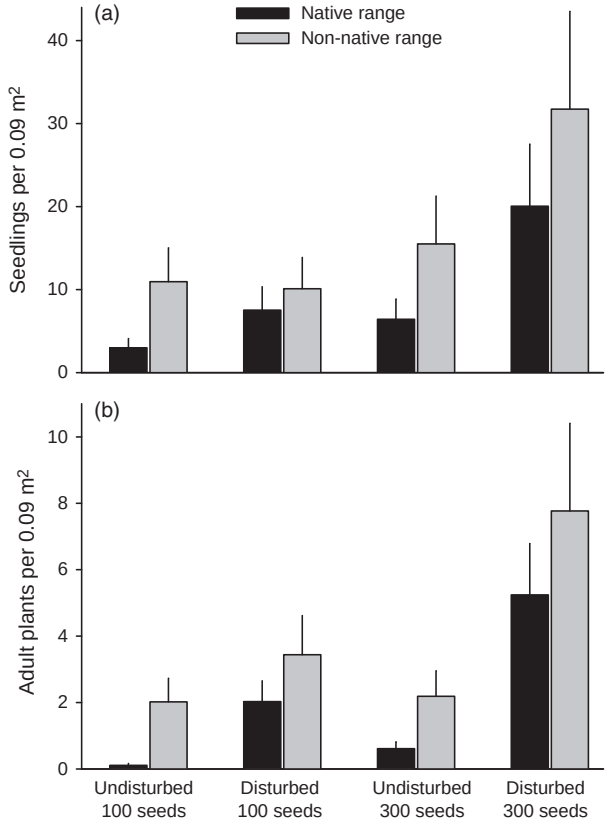

Fig. 2. Least squared means (+SEM) (a) for the cumulative number of knapweed seedlings that recruited into 0.3 × 0.3 m plots and (b) number of adult plants in plots at the end of the experiment as a function of disturbance and propagule pressure (100 or 300 knapweed seeds added to each plot). LS means were calculated from continent × disturbance × propagule pressure interactions reported in Table 2.

where we originally added 100 knapweed seeds was 1.1 (mean flowering = 0.45 ± 0.1) compared to a mean of 2.73 (mean flowering = 0.96 ± 0.14) in the plots with 300 seeds added. The effect of disturbance on the final number of adult plants (and number of flowering plants) was relatively weak in the non-native range compared to the native range (significant continent × disturbance interaction, Table 2 and Fig. 2b), particularly under low compared to high propagule pressure (continent × disturbance × propagule pressure interaction: $P < 0.04$, Table 2 and Fig. 2b). In other words, in the non-native range, disturbance only elevated adult abundance (and the number of flowering plants) under high propagule pressure (similar to the trend seen for seedling recruitment; Fig. 2a,b). Propagule pressure itself had a weaker positive effect on adult plant establishment in the non-native compared to the native range (continent × propagule pressure interaction: $P = 0.014$, Table 2 and Fig. 2b), but again, this pattern was primarily evident in disturbed relative to undisturbed plots as indicated by the significant three-way interaction (Table 2 and Fig. 2b).

Discussion

Our goal was to determine whether soil fungal pathogens, soil disturbance and propagule pressure could singly or in combination help explain the greater abundance of knapweed in the introduced versus the native range. We found no evidence that soil fungal pathogens explained lower knapweed abundance in Europe than Montana. Interestingly, neither did we find that small-scale disturbance or increasing propagule pressure differentially led to greater knapweed recruitment or abundance in Montana compared to Europe. However, we did find two large biogeographic effects. First, knapweed recruited more abundantly, and ultimately achieved greater adult abundance, in undisturbed plots in North America compared to Europe. For number of adults, this was particularly evident in low seed addition plots. Second, a much higher proportion of knapweed seeds germinated in bags buried at Montana sites compared to European sites. Although disturbance and increasing seed input (i.e. propagule pressure) both positively enhanced knapweed recruitment and establishment, these effects were not generally greater in Montana than in Europe, as one would expect if they explained knapweed success as an exotic.

Within recipient communities, both propagule pressure and disturbance can enhance exotic abundance (Burke & Grime 1996; D'Antonio, Dudley & Mack 1999; Mack *et al.* 2000; Parker 2001; Kellogg & Bridgham 2004; Paiaro, Mangeaud

& Pucheta 2007; Britton-Simmons & Abbott 2008; Simberloff 2009). We also found that these factors increased the magnitude of knapweed seedling recruitment and adult plant establishment in Montana (Fig. 1). However, for these factors to explain knapweed's dominance as an exotic (and its lower abundance where native), either rates of disturbance and/or the degree of propagule pressure must be higher in the introduced than the native range, or knapweed must have a greater positive response to these factors where it has been introduced compared to where it is native. Although we have not quantified natural rates of disturbance in Montana versus Europe, we can see no *a priori* reason why rates of disturbance should be substantially higher in Montana than in Europe. Thus, the key question is whether knapweed responds more favourably to disturbance (or propagule pressure) in Montana than in Europe. We found no support for this. Had we only performed these experiments within the introduced range, which is typical, we might have concluded that propagule pressure and/or disturbance was driving invasion success, when in fact we did not find any evidence for this in the conditions of our sites, timeframe and scale of our treatments.

It is interesting to note that in our experiments, even though increasing propagule pressure produced more knapweed seedlings, the percentage of seeds that produced seedlings in 100 and 300 seed addition treatments did not vary substantially. In the invasion biology literature, many studies have examined how the number of introductions, or the size of introductions, might increase the probability of exotic establishment (Simberloff 2009). In most of these cases, the question concerns what minimum level of propagule pressure is needed to overcome environmental or demographic stochasticity such that a new species can simply establish in a new range. In this work, the assumption is that the probability of successful establishment increases with propagule pressure. However, small scale experiments to test the role of propagule pressure in plants usually only involve a 'zero seeds added' and a '+seeds added' treatment (but see Britton-Simmons & Abbott 2008). These experiments ask whether local establishment might be limited due to the lack of supply of exotic propagules (Simberloff 2009). Since seed inputs are not manipulated across a range of densities, it is unclear how the probability of successful establishment changes with propagule pressure. Moreover, since almost all studies of propagule pressure are conducted in the introduced range only (but see Hierro *et al.* 2006; Williams, Auge & Maron 2010) the question of whether there are biogeographic differences in either the percentage of seeds that successfully establish or the total number of seedlings that establish as a function propagule pressure cannot be tested. In our experiment, we did not find a substantial difference between Europe and North America in how propagule pressure influenced cumulative seedling recruitment, although propagule pressure and continent did have interactive effects on the final number of adult plants and the number of flowering plants. The fact that we found slightly less recruitment (as a percentage of seeds added) in high seed addition plots versus low suggests that at some upper bound of seed addition, negative density dependence begins to compensate for increased seed rain. Cumulative seedling recruitment and the final number of adult plants in undisturbed plots were greater in Montana than in Europe, and the number of adults was greater across disturbance, fungicide and propagule pressure treatments in Montana than Europe. One explanation for this is that in European grasslands, knapweed suffers from more intense interspecific competition than it does in drier grasslands in Montana (Callaway *et al.* 2011b). The harsher competitive environment for adult plants in Europe likely also inhibits recruitment and establishment of knapweed in Europe compared to Montana. This is consistent with a recent common garden demography study, which showed that increased establishment rates for plants from North American versus European knapweed populations may underlie their higher population growth rates (Hahn, Buckley & Müller-Schärer 2012).

While knapweed tended to establish at greater abundance in undisturbed plots in Montana versus Europe, and indeed, across all treatments knapweed establishment was greater in Montana compared to Europe, the absolute difference in establishment between continents was not dramatic (Fig. 1b). One possible explanation for this may be due to our selection of experimental sites. In Montana, we only established experiments in sites where knapweed occurred at low-to-moderate densities, so that plant density at sites in Europe and Montana would be similar. Yet, this conservative approach might have resulted in our picking sites in Montana that were in some way inherently difficult for knapweed to reach high densities.

We originally hypothesized that we might observe strong negative effects of soil pathogens on knapweed recruitment or establishment in Europe. This expectation was based on results from previous soil feedback experiments that have found strong negative effects of European soils on knapweed performance compared to North American soils (Callaway *et al.* 2004a) and other field experiments showing that a different fungicide shifted competitive advantages away from knapweed and in favour of some native species (Callaway *et al.* 2004b). Moreover, other studies have found that soil biota can have more negative effects on invasive plants in their native ranges than in their non-native ranges (Reinhart & Callaway 2006; Kulmatiski *et al.* 2008; Callaway *et al.* 2011a). However, despite the fact that our fungicide treatment suppressed soil fungal biomass, and despite the fact that other experiments have shown that the same fungicides as we applied, at the same application rate, could reduce pathogenic root lesions and increase plant production in experiments involving native plants (Maron *et al.* 2011), we found no evidence for this treatment positively influencing either seed germination, seedling recruitment or establishment in Europe. To the contrary, we found that fungicide treatment reduced knapweed establishment. If fungal soil pathogens in Europe attack species in a density-dependent manner, we may not have found strong positive effects simply because knapweed occurred at only moderate background densities at our experimental sites. The negative impact of fungicide treatment on knapweed establishment in Europe is puzzling to us, and our only explanation is speculative. One possibility is that fungicide application

reduced pathogen attack on surrounding natives, thereby enhancing their productivity and creating a more competitive environment for knapweed. While some studies have found relatively high rates of pathogen attack on exotics in recipient communities (Parker & Gilbert 2007), we did not find such effects.

The use of fungicides to suppress pathogenic soil fungi is not without potential problems. For example, we cannot be certain that the fungicides we applied effectively suppressed all those soil pathogens that might be specific to knapweed. As well, although we found no non-target effects of fungicides on AMF infectivity and total fungal and bacterial biomass in the soil, we cannot be certain that these fungicides did not influence other attributes of the soil biota. However, in a greenhouse experiment where we grew knapweed in sterile soil which we either treated with fungicides (at the same concentrations per unit area we used in the field) or not, we saw no effects of fungicide application on knapweed biomass (J. L. Maron and R. M. Callaway, unpublished data). Beyond these results, we note that the main hypothesis we wished to test concerned whether fungal soil pathogens had more negative impacts on knapweed recruitment in Europe than in North America. While we generally did not see such effects, for non-target effects of fungicides to be problematic in the context such an experiment, they would have: (i) had to have large non-target effects that translated to significant alterations in knapweed recruitment or survival *and* (ii) these non-target impacts would have had to be different across ranges.

One intriguing biogeographic pattern we found is significantly higher seed germination of knapweed in buried bags in Montana compared to Europe. Because we could not control for environmental differences between Europe and Montana, we are unsure whether this difference in germination rate is an environmental effect, a maternal effect or the result of some rapid evolutionary change that knapweed has undergone post-introduction. However, in a common greenhouse environment Ridenour *et al.* (2008) showed that germination rates of knapweed seeds collected in the introduced range were substantially higher than the germination rate of seeds collected in the native range. Whether maternal effects or rapid evolutionary changes within North America underlie this biogeographic difference in germination rate remains unclear. Founder effects/genetic drift are unlikely explanations for our results since it appears that there have been multiple introductions of *C. stoebe* into North America (Marrs, Sforza & Huffbauer 2008). Regardless of the cause, the fact that knapweed seeds in Europe tend to possess greater dormancy than in North America has implications for population growth rates. All else being equal, population growth of knapweed in Montana should be enhanced compared to Europe due to the reduced dormancy of knapweed seeds.

In the invasion literature there has been much discussion as to whether certain species are 'passengers' or 'drivers' of invasion (MacDougall & Turkington 2005). The distinction between these is that 'passengers' capitalize on disturbance to invade systems, whereas 'drivers' can invade undisturbed communities and have large impacts. Our results are consistent with the notion that knapweed in Montana may be a 'driver', in that it can recruit into undisturbed sites and once established at high density it can have large negative impacts on the native community (Maron & Marler 2008a,b). Moreover, it is interesting to note that knapweed recruited and established in undisturbed non-native sites more abundantly than in undisturbed native sites, a pattern is consistent with apparent differences in competitive resistance of natives to knapweed between the ranges (Callaway *et al.* 2011b) and the idea that general competitive interactions contribute to the striking dominance of knapweed in some parts of North America.

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References

Afek, U., Menge, J.A. & Johnson, E.L.V. (1990) Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion, and pepper. *Plant Disease*, **74**, 117–120.

Bell, T., Freckleton, R.P. & Lewis, O.T. (2006) Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, **9**, 569–574.

Bever, J.D. (1994) Feedback between plants and their soil communities in an old field community. *Ecology*, **75**, 1965–1977.

Britton-Simmons, K.H. & Abbott, K.C. (2008) Short- and long-term effects of disturbance and propagule pressure on a biological invasion. *Journal of Ecology*, **96**, 68–77.

Brundrett, M.C., Piché, Y. & Peterson, R.L. (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany*, **62**, 2128–2134.

Burke, M.J.W. & Grime, J.P. (1996) An experimental study of plant community invasibility. *Ecology*, **77**, 776–790.

Callaway, R.M. & Aschehoug, E.T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, **290**, 521–523.

Callaway, R.M., Thelen, G.C., Rodriquez, A. & Holben, W.E. (2004a) Soil biota and exotic plant invasion. *Nature*, **427**, 731–733.

Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W. & Gannon, J.E. (2004b) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology*, **85**, 1062–1071.

Callaway, R.M., Bedmar, E.J., Reinhart, K.O., Silvan, C.G. & Klironomos, J. (2011a) Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. *Ecology*, **92**, 1027–1035.

Callaway, R.M., Waller, L.P., Diaconu, A., Pal, R., Collins, A.R., Müller-Schärer, H. & Maron, J.L. (2011b) Escape from competition: neighbors reduce *C. stoebe* performance at home but not away. *Ecology*, **92**, 2208–2213.

Cycón, M., Wójcik, M. & Piotrowska-Seget, Z. (2011) Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil. *Biodegradation*, **22**, 573–583.

D'Antonio, C.M., Dudley, T.L. & Mack, M. (1999) Disturbance and biological invasions: direct effects and feedbacks. *Ecosystems of the World 16: Ecosystems of Disturbed Ground* (ed. L.R. Walker), pp. 413–452. Elsevier, Amsterdam.

Demanau, J., Monkiédjé, A., Njiné, T., Foto, S.M., Nola, M., Zebaze Togouer, S.H. & Kemka, N. (2004) Changes in soil chemical properties and microbial activities in response to fungicide Ridomil Gold plus copper. *International Journal of Environmental Research and Public Health*, **1**, 26–34.

DeWalt, S.J., Denslow, J.S. & Ickes, K. (2004) Natural enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology*, **85**, 471–483.
Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants*. Methuen, London.
Gurevitch, J., Fox, G.A., Wardle, G.M., Inderjit & Taub, D. (2011) Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters*, **14**, 407–418.
Hahn, M.A., Buckley, Y.M. & Müller-Schärer, H. (2012) Increased population growth rate in invasive polyploidy *Centaurea stoebe* in a common garden. *Ecology Letters*, **15**, 947–954.
van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M.J. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.
Hierro, J., Maron, J.L. & Callaway, R.M. (2005) A biogeographical approach to plant invasion biology: the importance of studying exotics in their introduced *and* native range. *Journal of Ecology*, **93**, 5–15.
Hierro, J.L., Villarreal, D., Eren, O., Graham, J.M. & Callaway, R.M. (2006) Disturbance facilitates invasion: the effects are stronger abroad than at home. *The American Naturalist*, **168**, 144–156.
Hobbs, R.J. (1989) The nature and effects of disturbance relative to invasions. *Biological Invasions: A Global Perspective* (eds J.A. Drake, H.A. Mooney, F. di Castri, R.H. Groves, F.J. Kruger, M. Rejmanek & M.H. Williamson), pp. 389–405. John Wiley and Sons, New York.
Hobbs, R.J. (1991) Disturbance as a precursor to weed invasion in native vegetation. *Plant Protection Quarterly*, **6**, 99–104.
Hobbs, R.J. & Huenneke, L.F. (1992) Disturbance, diversity, and invasion: implications for conservation. *Conservation Biology*, **6**, 324–337.
Jacobs, J.S., Sheley, R.L. & Cater, J.R. (2000) Picloram, fertilizer, and defoliation interactions on spotted knapweed reinvasion. *Journal of Range Management*, **53**, 309–314.
Kellogg, C.H. & Bridgham, S.D. (2004) Disturbance, herbivory, and propagule dispersal control dominance of an invasive grass. *Biological Invasions*, **6**, 319–329.
van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M. & Fischer, M. (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology Letters*, **13**, 947–958.
Klironomos, J.N. (1995) Arbuscular mycorrhizae of *Acer saccharum* in different soil types. *Canadian Journal of Botany*, **73**, 1824–1830.
Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.
Klironomos, J.N., Rillig, M.C. & Allen, M.F. (1999) Designing belowground field experiments with the help of semi-variance and power analyses. *Applied Soil Ecology*, **12**, 227–238.
Kulmatiski, A., Beard, K.H. & Stark, J.M. (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. *Journal of Applied Ecology*, **43**, 868–876.
Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M. (2008) Plant-soil feedbacks: a meta-analytical review. *Ecology Letters*, **11**, 980–992.
Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J. & Li, B. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, **177**, 706–714.
MacDonald, A.A.M. & Kotanen, P.M. (2010) The effects of disturbance and enemy exclusion on performance of an invasive species, common ragweed, in its native range. *Oecologia*, **162**, 977–986.
MacDougall, A.S. & Turkington, R. (2005) Are invasive species the drivers or passengers of change in degraded ecosystems. *Ecology*, **86**, 42–55.
Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.
Maron, J.L. & Marler, M. (2008a) Field based competitive impacts of invaders on natives at varying resource supply. *Journal of Ecology*, **96**, 1187–1197.
Maron, J.L. & Marler, M. (2008b) Effects of native species diversity and resource additions on invader impact. *The American Naturalist*, **172**, S18–S33.
Maron, J.L. & Vilà, M. (2001) Do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 363–373.
Maron, J.L., Marler, M., Klironomos, J. & Cleveland, C. (2011) Soil pathogens contribute to the positive plant diversity-productivity relationship. *Ecology Letters*, **14**, 36–41.
Marrs, R.A., Sforza, R. & Huffbauer, R.A. (2008) Evidence for multiple introductions of *Centaurea stoebe micranthus* (spotted knapweed, Asteraceae) to North America. *Molecular Ecology*, **17**, 4179–4208.
Mordecai, E.A. (2011) Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs*, **81**, 429–441.
Morris, S.J., Zink, T., Conners, K. & Allen, M.F. (1997) Comparison between fluorescein diacetate and differential fluorescent staining for determining fungal biomass in soils. *Applied Soil Ecology*, **6**, 161–167.
Mráz, P., Bourchier, R., Treier, U., Schaffner, U. & Müller-Schärer, H. (2011) Polyploidy in phenotypic space and invasion context: a morphometric study of *Centaurea stoebe*. *International Journal of Plant Sciences*, **172**, 386–402.
Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology and Evolution*, **19**, 417–422.
Ortega, Y.K. & Pearson, D.E. (2005) Weak vs. strong invaders of natural plant communities: assessing invisibility and impact. *Ecological Applications*, **15**, 651–661.
Ortega, Y.K. & Pearson, D.E. (2011) Long-term effects of weed control with picloram along a gradient of spotted knapweed invasion. *Rangeland Ecology and Management*, **64**, 67–77.
Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404**, 278–281.
Paiaro, V., Mangeaud, A. & Pucheta, E. (2007) Alien seedling recruitment in response to altitude and soil disturbance in the mountain grasslands of central Argentina. *Plant Ecology*, **193**, 279–291.
Parker, I.M. (2001) Safe site and seed limitation in *Cytisus scoparius* (Scotch broom): invasibility, disturbance, and the role of cryptogams in a glacial outwash prairie. *Biological Invasions*, **3**, 323–332.
Parker, I.M. & Gilbert, G.S. (2007) When there is no escape: the effects of natural enemies on native, invasive and noninvasive plants. *Ecology*, **88**, 1210–1224.
van der Putten, W.H., van Dijk, C. & Peters, B.A.M. (1993) Plant specific soil borne diseases contribute to succession in foredune vegetation. *Nature*, **362**, 53–56.
Reinhart, K.O. & Callaway, R.M. (2006) Soil biota and invasive plants. *New Phytologist*, **170**, 445–457.
Reinhart, K.O., Packer, A., van der Putten, W.H. & Clay, K. (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046–1050.
Reinhart, K.O., Royo, A.A., van der Putten, W.H. & Clay, K. (2005) Soil feedback and pathogen activity associated with *Prunus serotina* throughout its native range. *Journal of Ecology*, **93**, 890–898.
Ridenour, W.M. & Callaway, R.M. (2001) The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia*, **126**, 444–450.
Ridenour, W.M., Vivanco, J.M., Feng, Y., Horiouchi, J. & Callaway, R.M. (2008) No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. *Ecological Monographs*, **78**, 369–386.
Seymour, N.P., Thompson, J.P. & Fiske, M.L. (1994) Phytotoxicity of fosetyl-Al and phosphonic acid to maize during production of vesicular-arbuscular mycorrhizal inoculum. *Plant Disease*, **78**, 441–446.
Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology and Evolution*, **17**, 170–176.
Simberloff, D. (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 81–102.
Treier, U.A., Broennimann, O., Normand, S., Guisan, A., Schaffner, U., Steinger, T. & Müller-Schärer, H. (2009) Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology*, **90**, 1366–1377.
Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jaršők, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. & Pyšek, P. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702–708.
Watson, A.K. & Renney, A.J. (1974) The biology of Canadian weeds. *Centaurea diffusa* and *C. maculosa*. *Canadian Journal of Plant Science*, **54**, 687–701.
Williams, J.L., Auge, H. & Maron, J.L. (2010) Effects of disturbance and herbivory on invasive plant abundance at home and abroad. *Ecology*, **91**, 1355–1366.
Williamson, M. (1996) *Biological Invasions*. Chapman & Hall, London.